UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/563,195	04/03/2006	Alison Velyian Todd	22238.0004	6479

78989 7590 09/14/2011

Sullivan & Worcester LLP
1666 K Street, N.W.
Washington, DC 20006

EXAMINER	
BHAT, NARAYAN KAMESHWAR	
ART UNIT	PAPER NUMBER
1634	

NOTIFICATION DATE	DELIVERY MODE
09/14/2011	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

docketing@sandw.com
mdemattia@sandw.com
tdores@sandw.com

Office Action Summary	Application No.	Applicant(s)	
	10/563,195	TODD ET AL.	
	Examiner	Art Unit	
	NARAYAN BHAT	1634	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *28 February 2011*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

5)☒ Claim(s) *1-38* is/are pending in the application.

5a) Of the above claim(s) *38* is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *1-37* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☒ The drawing(s) filed on *03 January 2006* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

12)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

13)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/GB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

Continued Examination under 37 CFR 1.114

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on February 28, 2011 has been entered.

Claim Status

2. Claims 1-38 are pending in this application.

3. Claim 38 is withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected invention of group II there being no allowable generic or linking claim. Applicant timely traversed the restriction (election) requirement by telephone on May 18, 2007.

4. Claims 1-37 are under prosecution.

Withdrawn Objection

5. The previous objection of claim 15 in the office action dated September 1, 2010 has been withdrawn in view correctly identifying the status of the said claim.

Claim Rejections - 35 USC § 103

6. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

7. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

8. Claims 1-4, 14-31, 33 and 35-37 are rejected under 35 U.S.C. 103(a) as being unpatentable over Gitan et al (Genome Research, 2001, 12, 158-164, cited in the previous rejection) in view of Bransteitter et al (PNAS, 2003, 100, 4102-4107, cited in the IDS filed 4/3/2006, cited in the previous rejection).

Both Gitan and Bransteitter teach a method to determine the methylation status of the nucleic acid and therefore analogous arts.

The previous rejection is maintained.

Regarding claim 1, Gitan teaches a method for detecting the presence or level of alkylated cytosine in a sample of genomic double stranded DNA from an individual comprising following steps.

Regarding step 'a', Gitan teaches obtaining a sample of the genomic double stranded DNA from the individual (pg. 162, Methods section, pg. 162, column 2, and paragraph 3).

Regarding step 'b', Gitan teaches converting at least one region of the double stranded DNA to single stranded DNA by treating with mild heat and alkali/sodium bisulfite using Intergen kit (Gitan, pg. 162, column 2, paragraph 4, Intergen kit, pg. 6, Modification protocol, pg. 7. step II).

Regarding step 'c', Gitan teaches that the single stranded DNA comprises both methylated (i.e., alkylated) and unmethylated cytosine and bisulfite treatment converts unmethylated cytosine to uracil, whereas methylated cytosine remain unchanged in the single stranded DNA (Fig. 1, pg. 159, column 1, Results section, paragraph 1), which encompasses differentially modifying alkylated cytosine and cytosine present in the single stranded DNA. Methylated cytosine of Gitan is the alkylated cytosine as recited in instant claim 35. Gitan does not teach an enzyme differentially modifying alkylated cytosine and cytosine present in the single stranded DNA.

Regarding step 'd', Gitan teaches determining the level of bisulfite modification of the target region by analyzing converted (i.e., unmethylated) and unconverted (i.e., methylated alleles) alleles on an oligonucleotide array (Fig. 1, bottom panel, pg. 159, results section paragraph 1).

However, an enzyme differentially modifying alkylated cytosine and cytosine was known in the art at the time the claimed invention was made as taught by Bransteitter.

Bransteitter teaches cytidine deaminase activity assay based on the detection of conversion (or lack thereof) of cytidine to uracil and further teaches that if non-methylated cytidine is present in the nucleic acid sequence targeted, it is deaminated. Bransteitter also teaches that the cytidine deaminase enzyme modulates the activity of single strand DNA comprising cytosine and methylated cytosine differentially by 10-fold and does not convert cytidine into uracil if the DNA is in double stranded form (Fig. 4b, Abstract, Table 1 and pg. 4106, column 1, paragraph 4). One having ordinary skill in the art would recognize that cytidine activity assay comprising cytidine deaminase and specifically detecting the methylation status of cytidine in the single stranded nucleic acid sequence of Bransteitter encompass a method for detecting the presence of alkylated (i.e., methylated) cytosine. Bransteitter specifically teach that AID and further teaches that the deamination is very rapid and completes in minutes (Fig. 4A, pg. 4106, column 1, last paragraph).

As discussed above, both Gitan and Bransteitter teach a method steps for detecting the methylation and therefore are steps are combinable. Gitan teaches that the bisulfite treatment take long hours to differentially modulate the single stranded DNA comprising unmethylated and methylated cytosine, whereas Bransteitter teaches that an enzyme treatment takes minutes to differentially modulate single stranded DNA comprising unmethylated and methylated cytosine (or cytidine), thus providing motivation to one having ordinary skill in the art to use an enzyme for detecting

methylation status in a target genome, because it requires less time and does not require toxic chemical such as bisulfite and longtime to process the reaction. Therefore one of ordinary skill in the art would have recognized the advantage using enzymatic deamination rather than chemically induced one.

It would have been prima facie obvious to one having ordinary skill in the art at the time the claimed invention was made to modify the bisulfite deamination step of Gitan with the deamination step using cytidine deaminase of Bransteitter with a reasonable expectation of success with the expected benefit of using cytidine deaminase enzyme for detecting methylation status of the target genome in minutes as taught by Bransteitter (Fig. 4A and 4B, pg. 4106, column 1, paragraph 4), thus expediting the detection of methylation pattern of cancer causing genes using the methylation specific oligonucleotide microarray of Gitan. One having ordinary skill in the art would have reasonable expectation of success because it merely involves substitution of a chemical deamination step with an enzymatic deamination step, which is routinely practiced in the art as exemplified by Bransteitter.

It is further noted that in KSR, the Supreme Court particularly emphasized "the need for caution in granting a patent based on the combination of elements found in the prior art," (USPQ2d at 1395), and reaffirmed principles based on its precedent that "[t]he combination of familiar elements according to known methods is likely to be obvious when it does no more than yield predictable results." In the instant case, the steps of chemical deamination and enzymatic deamination are very familiar steps routinely practiced in the art. As discussed above, one having ordinary skill in the art would

recognize the advantage of substituting the quicker enzymatic deamination step of Bransteitter rather than chemically induced deamination step of Gitan and thus are known to produce the expected results of detecting the presence of alkylated cytosine. Therefore, the method steps of claim 1are obvious over Gitan in view of Bransteitter.

The teachings of Gitan in view of Bransteitter regarding dependent claims 2-4, 14-31, 33 and 35-37 are discussed below.

Regarding claims 2 and 3, Gitan teaches that the single stranded DNA is reacted with the bisulfite under conditions such that the bisulfite reacts substantially only with cytosine (Fig. 1, pg. 159, column 1, Results section, paragraph 1). As discussed above Bransteitter teaches the step of single stranded DNA reacting with the enzyme under conditions such that the enzyme reacts substantially only with the unmethylated cytosine.

Regarding claim 4, Gitan teaches that the conversion of the region of the double stranded DNA to the single stranded DNA comprises separating the two strands of the double stranded DNA by treating with mild heat and alkali/sodium bisulfite using Intergen kit (Gitan, pg. 162, column 2, paragraph 4, Intergen kit, pg. 6, Modification protocol, pg. 7. step II). It is also noted that conversion of double stranded DNA to the single stranded DNA are routinely practiced in the art.

Regarding claim 14, Gitan teaches that the determination of the level of bisulfite modification of the single stranded DNA with single stranded comprises analyzing for methylated and unmethylated cytosine sequence variations arising from the

modification of the target region of the single stranded DNA by the bisulfite (Fig. 4, see the legend).

Regarding claim 15, Gitan teaches the PCR amplification of the selected target region using PCR to obtain an amplified product and analyzing the amplified product by sequence variation (Fig. 1, See the legend for details).

Regarding claim 16, Gitan teaches that the analysis of the amplified product comprises subjecting the amplified product to a technique involving the use of probes that bind to specific nucleic acid sequences (Fig. 1, bottom panel).

Regarding claim 17, Gitan teaches that the analysis of the amplified product comprises subjecting the amplified product to a polymerase chain reaction technique with nested primers (pg. 162, column 1, and paragraph 1).

Regarding claim 18, Bransteitter et al teaches that AID enzyme deaminates cytosine in the target region of the single stranded DNA (Fig. 1, single strand DNA labeled as ssDNA).

Regarding claim 19, Bransteitter et al teaches that AID enzyme and RNaseA (i.e., combination of enzymes) are employed to differentially modify 5-methyl cytosine (i.e., alkylated cytosine) and cytosine in the target region (Fig. 4B, pg. 4106, column 1, and last paragraph).

Regarding claims 20 and 21, Bransteitter et al teaches that the AID enzyme is a deaminase enzyme having deaminase activity (Fig. 1).

Regarding claim 22, Gitan teaches detecting the presence of methylated cytosine in an ER alpha gene (Fig. 4).

Regarding claim 23, Gitan teaches detecting the presence of methylated cytosine in a promoter region, i.e., "5' untranslated region of a gene" (pg. 162, column 1, paragraph 1, lines 1-2).

Regarding claim 24, Gitan teaches that the level of methylated cytosine comprises hypermethylation (Figs. 4 and 5 and pg. 160, column 1, paragraph 2).

Regarding claim 25, Gitan teaches the levels of methylated cytosine in the ER alpha gene is lower in MCF-7 cells than in the MDA-MB-231 cells (Fig. 4, pg. 160, column 1, paragraph 2), which encompasses the levels of methylated cytosine comprise hypomethylation.

Regarding claim 26, Gitan teaches that the gene is ER alpha (Abstract).

Regarding claims 27-31, Gitan teaches detection of altered level of alkylated cytosine in the target region of the single stranded DNA is a marker for cancer (Abstract and pg. 161, column 1, paragraph 2).

Regarding claim 33, Gitan teaches that the variable methylation of the ER alpha CpG island in normal fibroblast strain HFF results in silencing of the ER alpha gene expression (Fig. 5, pg. 160, column 1, last paragraph and pg. 161, column 1, first paragraph), which encompasses the level of methylated cytosine indicating the absence of an altered gene imprinting state.

Regarding claim 35 and 36, Gitan teaches that the alkylated cytosine is 5- methyl cytosine (pg. 158, column 2, lines 1-2 and pg. 163, column 2, reference of Frommer et al).

Regarding claim 37, Gitan teaches that the double stranded DNA is a genomic DNA (pg. 162, Method section, and paragraph 1).

9. Claims 5-13 are rejected under 35 U.S.C. 103(a) as being unpatentable over Gitan et al (Genome Research, 2001, 12, 158-164, cited in the previous rejection) in view of Bransteitter et al (PNAS, 2003, 100, 4102-4107, cited in the IDS filed 4/3/2006, cited in the previous rejection)as applied to claims 1 and 4 as above and further in view of Kuhn et al (J. Am. Chem. Soc., 2002, 124, 1097-1103, cited in the previous office action).

Gitan, Bransteitter and Kuhn teach a method for nucleic acid detection and therefore are analogous arts.

The previous rejection is maintained.

The teachings of Gitan and Bransteitter regarding claims 1 and 4 are described above in section 8.

Regarding claims 5-13, Bransteitter teaches generating a bubble comprising single stranded region in the double stranded region using partially complementary double stranded DNA (Table 1, legend). Gitan and Bransteitter do not teach using strand displacing probes to separate the two strands of the double stranded DNA. However, partially separating the two strands of the double stranded DNA with strand displacing probes were known in the art at the time the claimed invention was made as taught by Kuhn.

Regarding claim 4, Kuhn teaches a method for opening of the double stranded DNA with PNA openers to partially separate the two strands of the double stranded DNA (Fig. 5A, right panel, pg. 1101, column 1, and paragraph 2).

Regarding claim 5, Kuhn teaches a method wherein PNA openers (i.e., strand displacing probes) are used to partially separate the two strands of the double stranded DNA (Fig. 5A, right panel, pg. 1101, column 1, paragraph 2).

Regarding claim 6, Kuhn teaches that the strand displacing probes are PNA containing probes (Fig. 5A, right panel, pg. 1101, column 1, and paragraph 2).

Regarding claim 7, Kuhn teaches a method wherein the double stranded DNA is opened with PNA openers and further teaches that PNA openers forms triplexes and exposes the displaced DNA strand for binding with other DNA and PNA beacons (Fig. 5A and B, right panel, pg. 1101, column 1, paragraph 2), thus teaching inhibiting annealing of the two strands of the double stranded DNA together once they have been separated to facilitate access to the target region by the enzyme. With regard to the recitation of "to facilitate access to the target region by the enzyme", the phrase is the property of the DNA being at least partially separated, which Kuhn teaches.

Regarding claim 8, Kuhn teaches hybridizing at least one PNA beacon probe with a strand of the double stranded DNA following separation of the two strands to form a triplex structure, thereby inhibit the annealing of the two strands together (Fig. 5A and B, right panel, pg. 1101, column 1, and paragraph 2).

Regarding claim 9, Kuhn teaches that the PNA probe is antisense probe (Fig. 5B, right panel, Fig. 7A, See the legend, pg. 1102, column 2, paragraph 1).

Regarding claim 10, Kuhn teaches at least two PNA opener probes are hybridized with the strand of the double stranded DNA, one of the probes hybridizing with a region of the strand downstream of the target region (Fig. 5A, right panel, see the PNA opener at the right side of the displaced strand) and other probe hybridizing with a region of the strand upstream of the target region (Fig. 5A, right panel, see the PNA opener at the left side of the displaced strand).

Regarding claim 11, Kuhn teaches wherein the probe hybridizes with upstream and downstream regions of the strand which flank the target region such that a loop which incorporates the target region is formed in the strand (Fig. 5A, right panel, see the loop formed by two PNA openers).

Regarding claim 12, Kuhn teaches that the probe hybridizes with the strand of the double stranded DNA either side of the target region (Fig. 5A, right panel) and further teaches that the probe has a middle region of non-complementary sequence that does not hybridize with the target region such that a loop incorporating the target region is formed in the strand (Fig. 5A, right panel).

Regarding claim 13, Kuhn teaches that the middle region of the probe incorporates inverted repeats that hybridize together following hybridization of the probe with the strand of the double stranded DNA (Fig. 5A, right panel).

Kuhn also teaches that PNA beacons are insensitive to the presence of salt and DNA-binding/processing proteins and have a great potential as robust tools for recognition of specific sequence within double strand DNA without denaturation and deproteinization of duplex DNA (Abstract).

It would have been prima facie obvious to one having ordinary skill in the art at the time the claimed invention was made to modify the step of converting double stranded DNA into single stranded DNA of Gitan with the PNA openers of Kuhn with a reasonable expectation of success with the expected benefit of using PNA beacons, which are insensitive to the presence of salt and DNA-binding/processing proteins and have a great potential as robust tools for recognition of specific sequence within double strand DNA without denaturation and deproteinization of duplex DNA as taught by Kuhn (Abstract), thereby opening the double stranded DNA (i.e., generating the bubble) at any desired sequence of the DNA.

10. Claim 32 is rejected under 35 U.S.C. 103(a) as being unpatentable over Gitan et al (Genome Research, 2001, 12, 158-164, cited in the previous rejection) in view of Bransteitter et al (PNAS, 2003, 100, 4102-4107, cited in the IDS filed 4/3/2006, cited in the previous rejection) as applied to claim 1 as above and further in view of Opdecamp et al (Nucleic Acids Research, 1992, 20, 171-178, cited in the previous office action).

Gitan, Bransteitter and Opdecamp teach a method for manipulating DNA and therefore are analogous arts.

The previous rejection is maintained.

The teachings of Gitan and Bransteitter regarding claim 1 are described above in section 8.

Regarding claim 32, Gitan teaches the presence of methylated cytosine in human foreskin fibroblast (Fig. 5). Gitan and Bransteitter do not teach levels of

methylated cytosine to indicate the presence or absence of fetal cells. However, levels of methylated cytosine to indicate the presence or absence of fetal cells were known in the art at the time the invention was made as taught by Opdecamp.

Opdecamp teaches a method for detecting the presence or level of alkylated cytosine in a sample of genomic double stranded DNA from an individual, the method further comprising the detection of an altered level of alkylated cytosine in fetal liver than in adult liver (Fig. 5, Compare the pattern of fetal and adult liver), which encompasses the level of the alkylated cytosine to indicate the presence of fetal DNA.

Opdecamp also teaches that higher gene expression in fetal than in adult cells is due to methylation pattern of unmethylated site in the non-coding portion of the gene in fetal and adult cells (Abstract).

It would have been prima facie obvious to one having ordinary skill in the art at the time the claimed invention was made to apply the method of detecting methylation pattern in cancer cells of Gitan to detect the presence of fetal cells as suggested by Opdecamp with a reasonable expectation of success with the expected benefit of detecting genes that are expressed in fetal cells are also expressed in cancer cells as taught by Opdecamp (Abstract).

11. Claim 34 is rejected under 35 U.S.C. 103(a) as being unpatentable over Gitan et al (Genome Research, 2001, 12, 158-164, cited in the previous rejection) in view of Bransteitter et al (PNAS, 2003, 100, 4102-4107, cited in the IDS filed 4/3/2006, cited in

the previous rejection) as applied to claim 1 as above and further in view of in view of Paulson et al (J. Virol., 1999, 73, 9959-9968, cited in the previous office action).

Gitan, Bransteitter and Paulson teach a method for manipulating DNA and therefore are analogous arts.

The previous rejection is maintained.

Claim 34 is dependent from claim 1. The teachings of Gitan and Bransteitter regarding claim 1 are described above in section 8.

Regarding claim 34, Gitan teaches that the methylated cytosine in multiple CpG island loci changes in breast cancer (Abstract). Gitan and Bransteitter do not teach that the presence of alkylated cytosine for indicating the presence or absence of a pathogen or a microorganism.

However, the presence of the alkylated cytosine indicating the presence or absence of a pathogen or a microorganism was known in the art at the time the invention was made as taught by Paulson.

Paulson teaches that the EBV is the etiologic agent of infectious mononucleosis, i.e., a "pathogen" (pg. 9959, column 1, paragraph 1) and further teaches that the presence of EBV is characterized by the presence of methylation at its promoter sites (Fig. 3, pg. 9964, column 2 paragraph 2). Paulson also teaches that the EBV usurps the host cell directed methylation system to regulate pathogen gene expression and thereby establish a chronic infection (Abstract).

It would have been prima facie obvious to one having the ordinary skill in the art at the time the claimed invention was made to apply the method of detecting

methylation pattern in cancer cells of Gitan for detecting the presence of pathogen as suggested by Paulson et al with a reasonable expectation of success with the expected benefit of detecting expression of genes that are induced by pathogen EBV as taught by Paulson et al (Abstract, pg. 9959, column 1, and paragraph 1) are also induced or repressed in cancer cells.

Response to Remarks from the Applicant

Rejections under 35 U.S.C. § 103(a)

12. Applicant's arguments filed February with respect to claims 1-4, 14-31, 33 and 35-37 as being unpatentable over Gitan in view of Bransteitter have been fully considered (Remarks, pgs. 8-10) but they are not persuasive for the following reasons.

Applicant's argues that Bransteitter is not "interested in understanding the importance of methylation patterns". Rather, Bransteitter is clearly concerned only with class switch recombination and somatic hypermutation in B cells (see abstract) In particular, Bransteitter identifies a role for AID in SHM which is noted by the authors to be a process "which occurs only in the presence of RNA transcription" and which the present Applicant notes occurs only in B-cells. Thus it is erroneous to argue that Bransteitter is concerned with the importance of methylation patterns. The fact that AID deaminates unmethylated cytosine was identified only as part of the elucidation of the mechanism by which this enzyme works in SHM. Accordingly the Applicant submits that the Examiner's statement that both Gitan and Bransteitter are "interested in the importance of methylation pattern in biological processes" is incorrect and fails to

establish any motivation to combine the method steps of Gitan with Bransteitter (Remarks, pg. 9, paragraph 1).

These arguments are not persuasive because as discussed in section 8 above, Bransteitter teaches cytidine deaminase activity assay is based on the detection of conversion (or lack thereof) of cytidine to uracil and further teaches that if non-methylated cytidine is present in the nucleic acid sequence targeted, it is deaminated. Bransteitter also teaches that the cytidine deaminase enzyme modulates the activity of single strand DNA comprising cytosine and methylated cytosine differentially by 10-fold and does not convert cytidine into uracil if the DNA is in double stranded form (Fig. 4b, Abstract, Table 1 and pg. 4106, column 1, paragraph 4). One having ordinary skill in the art would recognize that cytidine activity assay comprising cytidine deaminase and specifically detecting the methylation status of cytidine in the single stranded nucleic acid sequence of Bransteitter encompass a method for detecting the presence of alkylated (i.e., methylated) cytosine.

Furthermore, it is noted that the MPEP 2111.03 makes it clear that the transitional term "comprising" is open-ended and does not exclude additional, unrecited elements (See, e.g., > Mars Inc. v. H.J. Heinz Co., 377 F.3d 1369,1376, 71 USPQ2d 1837, 1843 (Fed. Cir. 2004). Also the term "comprising" is a term of art used in claim language which means that the named elements are essential, but other elements may be added and still form a construct within the scope of the claim (Genentech, Inc. v. Chiron Corp., 112 F.3d 495, 501, 42 USPQ2d 1608, 1613 (Fed. Cir. 1997). In the instant case, as discussed above, in addition to class switch recombination and somatic

hypermutation, Bransteitter specifically teaches the method for detecting the presence of methylated cytosine and therefore arguments are not persuasive.

Furthermore, Applicant has not provided any support documents or declarations as to why one having ordinary skill in the art would not have used the method for cytidine deaminase activity which comprises a step for differentially detecting methylated versus unmethylated cytosine for detecting methylation in the single stranded DNA within minutes rather than a method of Gitan which requires the use of toxic chemicals and long hours .

Also, the MPEP 716.01(c) makes clear that "The arguments of counsel cannot take the place of evidence in the record. In re Schulze , 346 F.2d 600, 602, 145 USPQ 716, 718 (CCPA 1965). Examples of attorney statements which are not evidence and which must be supported by an appropriate affidavit or declaration include statements regarding unexpected results, commercial success, solution of a long - felt need, inoperability of the prior art, invention before the date of the reference, and allegations that the author(s) of the prior art derived the disclosed subject matter from the applicant." Here, the statements regarding the Bransteitter not teaching the method for detecting the alkylated cytosine (i.e., methylated cytosine) must be supported by evidence, not by argument.

Applicant further argues that Bransteitter is in no way concerned with detecting methylation patterns the Applicant submits that a skilled person would not have considered Bransteitter to be "reasonably pertinent" to the problem to be solved by the present application and therefore, would not have identified this reference or relied upon

its contents. In particular, the Applicant submits that Bransteitter relates solely to the field of somatic hypermutation and it is not at all pertinent to the problem addressed by the present specification namely the development of methods to measure methylation and overcome the disadvantages associated with bisulfite modification. Moreover, as argued below the skilled person reading Bransteitter is in fact taught that the activity of AID is limited to its role in SHM (Remarks, pg. 9, paragraph 3). These arguments are repetitive and are not persuasive for the same reasons as discussed above.

Applicant further argues that Bransteitter teaches away from the present claims because "SHM occurs only in the presence of RNA transcription" and requires RNASE Remarks, pg. 9, last paragraph and pg. 10, paragraph 1). Applicant's assertions are noted by the Examiner. However, as discussed above Bransteitter teaches the deamination of cytosine to uracil is single strand specific and the enzyme cytidine deaminase is ineffective in deaminating methylated cytosine (Table 1 and (Fig. 4B), thus teaching the broadly claimed method steps of requiring reacting a target region of the single stranded DNA from step (b) with at least one enzyme, the enzyme differentially modifying alkylated cytosine and cytosine present in the single stranded DNA (Emphasis added by the Examiner with an underline). For these reasons arguments based on the SHM are not persuasive.

Applicant's remaining arguments based on SHM, AID enzyme activity depends on the sequence context (Remarks, pg. 10, paragraph 2) are not persuasive in view of Bransteitter specifically teaching that the cytidine deaminase enzyme differentially modifies alkylated cytosine and cytosine present in the single stranded DNA.

Applicant further argues that Bransteitter teaches away from the use of single stranded DNA as the activity of the AID enzyme is far greater on partially single stranded DNA bubbles and the enzyme would not work in the absence of transcription (Remarks, pg. 10, paragraph 3). These arguments are not persuasive because Applicant has acknowledged that the AID enzyme (i.e., cytidine deaminase enzyme) is active on the single stranded DNA. Furthermore as discussed above claim 1 as recited with open claim language "comprising" can include other components. Therefore arguments regarding AID having the greatest activity on a 9 nucleotide bubble are not persuasive because instant claim 1 merely requires single stranded DNA, and the teachings of Bransteitter of a nucleic acid region comprising 9 nucleotide single stranded region meets that limitation.

Applicant further argues that the skilled person would, in view of Bransteitter, not consider the use of AID as a means to differentially modify alkylated cytosine and cytosine present in genomic or mitochondrial DNA based on the teachings of synthetic DNA of Bransteitter (Remarks, pg. 10, last paragraph). These arguments of counsel are not persuasive because as discussed above in section 8, the method steps of claim 1 are rejected over Gitan in view of Bransteitter. Gitan teaches the genomic DNA and denaturation step and the teachings of Bransteitter are relied for an enzyme that modulates the alkylated cytosine in the DNA differently than cytosine. Furthermore, Applicant has not provided any support documents or declarations as to why one having ordinary skill in the art would not substitute an enzymatic process for deamination,

which is faster and does not require harsh conditions for deamination. For these reasons arguments of counsel are not persuasive.

Applicant reiterates the arguments that the skilled person would not seek to use AID enzyme to investigate the methylation status of genomic DNA as AID enzymes were, at the time the present invention was made, known to be sequence specific. For example, the present specification as filed on page 8, line 1 to page 8, line 25 discusses the sequence specificity of AID enzymes. Accordingly a person skilled in the art looking to detect alkylated cytosine in DNA, rather than at specific sites within DNA, would not consider using AID as the sequence-specificity of that enzyme would be a disadvantage. In view of this the Applicant submits the skilled person before this present invention would not have used AID to modify unmethylated cytosine because the sequence specificity of AID would be understood to lead to inconsistent results (Remarks, pg. 11, paragraph 2). These arguments are repetitive and are not persuasive for the same reasons as discussed above.

Applicant further argues that that the modification of deamination step not obvious and further cites the composition of the denaturation buffer of Gitan would destroy the enzyme activity (Remarks, pg. 12, paragraph 3). Applicant's assertions are noted by the Examiner. However, arguments are not persuasive because one having ordinary skill in the art would recognize that part of using the Bransteitter protocol in the method of Gitan requires routine optimization that includes denaturation process that is conducive to the enzyme assay conditions.

Furthermore, the courts have ruled that where the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." In re Aller, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955). In the instant case general conditions for genomic DNA denaturation were known in the art of Gitan at the time the claimed invention was made. Therefore substituting a condition conducive to AID enzyme of Bransteitter would be a routine optimization process. Therefore arguments that Gitan's method is not compatible with the enzymatic deamination step of Bransteitter are not persuasive.

Applicant further argues that the present application solves different problems and further asserts that on page 4, line 2 to page 5, line 8 of the present specification as filed a further problem to be solved by the present invention is noted as the avoidance of artifacts which can arise in bisulfite based methods. Thus, the Applicant submits that the present invention also aims to solve the problem of artifacts of bisulfite modification. The Applicant respectfully submits that in light of this the combination of Gitan and Bransteitter is inappropriate as neither citation teaches or suggests the use of AID may be appropriate or useful to avoid the artifacts associated with bisulfite treatment (Remarks, pg. 13, paragraph 1). Applicant's alleged assertions are noted by the Examiner. Arguments are not persuasive because Bransteitter teaches that the AID enzyme deaminates alkylated cytosine and cytosine differently and within minutes (Fig. 4b), thus providing a motivation to one having ordinary skill in the art to use the AID enzyme rather than the chemical deamination, which requires long time and toxic chemicals.

In response to applicant's argument that the references fail to show certain features of applicant's invention, it is noted that the features upon which applicant relies (i.e., avoidance of artifacts in bisulfite based methods) are not recited in the rejected claim(s). Although the claims are interpreted in light of the specification, limitations from the specification are not read into the claims. See *In re Van Geuns*, 988 F.2d 1181, 26 USPQ2d 1057 (Fed. Cir. 1993). In the instant case, the method steps of claim 1 merely requiring the step of an enzyme which differentially modifies methylated cytosine and unmethylated cytosine in single stranded DNA is taught by Bransteitter and therefore arguments are not persuasive. Furthermore, Applicant has not provided any support documents or declaration about specific type of artifacts of the bisulfite solved by the instant application. Therefore, arguments of counsel without support documents of unexpected results are not persuasive.

Applicant further argues that the Examiner is using hindsight to make the combination of Gitan and Bransteitter. The Applicant submits that in full view of the claims the Examiner has merely searched for references which broadly disclose some of the steps of the claimed method (Gitan) and subsequently searched for another reference which allegedly provides the missing feature (Bransteitter). The Applicant argues that such hindsight analysis of the invention is impermissible and therefore the combination of Gitan and Bransteitter is also impermissible (Remarks, pg. 14, last paragraph).

In response to applicant's argument that the examiner's conclusion of obviousness is based upon improper hindsight reasoning, it must be recognized that

any judgment on obviousness is in a sense necessarily a reconstruction based upon hindsight reasoning. But so long as it takes into account only knowledge which was within the level of ordinary skill at the time the claimed invention was made, and does not include knowledge gleaned only from the applicant's disclosure, such a reconstruction is proper (See *In re McLaughlin*, 443 F.2d 1392, 170 USPQ 209 (CCPA 1971)). In the instant case as discussed above, the method steps of claim 1 are based on the knowledge gleaned from the references of Gitan and Bransteitter. Therefore arguments based on the impermissible hindsight are not persuasive.

Applicant's remaining arguments regarding the teachings of Kuhn, Opdecamp and Paulson are directed towards not curing the deficiency of Gitan et al and Bransteitter et al (Remarks, pgs. 14-15). These arguments are not persuasive because as described above and in section 8, Gitan in view of Bransteitter teach recited steps 'a' to 'd' and therefore it is maintained that the claimed method steps are obvious over Gitan in view of Bransteitter and the teachings of Kuhn are relied only for separating two strands of double stranded DNA with different means including strand displacing probes. Similarly the teachings of Opdecamp are relied only for the presence or level of the alkylated cytosine indicating the presence or absence of fetal DNA and the teachings of Paulson are relied only for the presence or level of the alkylated cytosine indicating the presence or absence of a pathogen.

Conclusion

13. No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Narayan K. Bhat whose telephone number is (571)-272-5540. The examiner can normally be reached on 8.30 am to 5 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Dave Nguyen can be reached on (571)-272-0731. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Narayan K. Bhat/

Examiner, Art Unit 1634

/Stephen Kapushoc/

Primary Examiner, Art Unit 1634